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Characteristics of the growth rate and lipid production in fourteen strains of Baltic green microalgae

by

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Abstract

Screening of fourteen Baltic green algal strains provided basic data on their mass culture potential for the purpose of valuable biomass production with particular emphasis on lipid content. Selected microalgae were grown under non-stressed conditions in order to identify those characterized by efficient lipid production. The tested strains exhibited significant differences in growth patterns and lipid yields. Strains belonging to *Chlorella* and *Stichococcus* genera exhibited the highest growth rates, ranging from 0.39 d^{-1} to 0.50 d^{-1} and thus the highest final cell density (> 10^7 cells ml^{-1}). Furthermore, five strains: *C. minutissima* BA-12, *C. fusca* BA-18, *C. vulgaris* BA-80, *Monoraphidium* sp. BA-165 and *Chlorella* sp. BA-167 were characterized by distinctively high lipid yield (> 60 mg l^{-1}). The same strains, together with *C. vulgaris* BA-02, were also shown as those with the highest volumetric lipid productivity, reaching > 30 mg l^{-1} d^{-1}. The tested Baltic strains performed well in terms of lipid production compared to the literature data, still leaving a great spectrum of opportunities for further lipid yield improvement.

Key words: lipids, lipid productivity, specific growth rate, green microalgae, Baltic

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Introduction

Cultures of microalgae gain increasingly more attention due to their high biomass productivity, which fits perfectly into the global trend of seeking new renewable energy and other desirable high value product sources (Spolaore et al. 2006). Rapid growth, the ability to accumulate large amounts of pigments, carbohydrates and lipids in cells are preferred features in the production of biofuels or natural bioactive compounds (Patterson et al. 1994). In addition, new materials offer many benefits for the environment, such as more efficient land use compared to terrestrial plants, CO_2 sequestration as well as the possibility of wastewater cleaning. Therefore, culturing microalgae on a larger scale can provide both economic and environmental benefits.

Large-scale production, in order to be profitable and undisturbed by external factors, requires proper optimization, which starts with the selection of suitable species and determination of their optimal growth conditions (Spolaore et al. 2006; Mata et al. 2010; Lam & Lee 2012; Tsarenko et al. 2016). It has been noted that rigorous selection for biotechnological use is challenging due to varying sets of characteristics between strains i.e. growth rate, cell size and the content of metabolism products (Griffiths & Harrison 2009). Depending on the purpose of mass culture and due to the fact that there are no strains satisfying all the demands, strains with a proper set of characteristics are selected. There are already strains indicated as having high potential to be used in mass cultures, i.e. species belonging to *Chlorella, Nannochloropsis, Scenedesmus* or *Botryococcus* genera (Mata et al. 2010; Sydney et al. 2011; Wuang et al. 2016). However, research in this area still requires further screening of strains isolated from natural waters, which will effectively grow under local environmental conditions.

Recently, more emphasis has been put on the content of high-energy compounds needed to increase the energy of biomass obtained from algae. Microalgae produce lipids as a storage material in the form of triglycerides (TAGs) or more complex lipids performing specific functions in the cell membranes (Khozin-Goldberg 2016). Studies have shown that the high cellular content of the desired product, such as triglycerides, in the case of biodiesel is not sufficient itself and must be accompanied by an adequate growth rate. In this respect, the algae to be selected should have a high growth rate and high lipid content during unstressed (exponential) growth (Borowitzka 2013).

The optimal growth conditions for several Baltic strains have been sufficiently described in the literature (e.g. Latała et al. 2006), although studies on their potential use in biotechnology are still in the initial phase. Culturing Baltic microalgae may be preferred because of their high adaptability to a relatively wide range of environmental conditions occurring in the Baltic Sea, e.g. relatively low salinity (8 PSU) and a wide range of temperature changes (0–2°C in winter and up to 22°C in summer). Recent screening tests of microalgae for valuable biomass production have shown that brackish Baltic strains performed equally well or even better in terms of biomass quality and yield compared to other strains, often used in algal research and development (Olofsson et al. 2015).

Therefore, this paper aims at comparing the growth rate and lipid production in fourteen selected strains of Baltic green microalgae. The strains were incubated under non-stressed conditions and their final cell count, growth rate as well as lipid yield and productivity were examined in order to identify those with inherent features of outstanding lipid production.

Materials and methods

The study included fourteen strains of green algae maintained as monocultures in the Culture Collection of Baltic Algae (CCBA; Latała et al. 2006) located at the Institute of Oceanography, University of Gdańsk (Table 1).

Microalgae were grown in 100 ml glass Erlenmeyer flasks filled with 50 ml of f/2 medium (Guillard 1975) prepared on the basis of artificial sea water (*Tropic Marine®*, Germany) with salinity of 8 PSU (similar to the salinity in the Gulf of Gdańsk) and autoclaved for 20 min at 121°C and 1.5 bar. Cultures were inoculated so as to obtain the initial cell density of 50 000 cell ml^{-1} and incubated for 21 days under constant light and temperature conditions, i.e. at 100 µmol m^2 s^{-1} in a 14 h:10 h light:dark cycle (white cool light was provided by fluorescent lamps Phillips 40W) and 18°C.

The volume of cells was calculated according to the instruction provided in Olenina et al. (2006).

The specific growth rates (µ; d^{-1}) of the tested strains were determined by counting cells using Bürker haemocytometer and calculated according to the equation provided by Guillard (1973):

$$M = (lnN_1 - lnN_0)t^{-1}$$

where N_0 and N_1 are the number of cells [cells ml^{-1}] at the beginning and at the end of the time interval, respectively, and Δt is the length of the time interval [days; d^{-1}].

Table 1

List of strains used in the study and their morphological characteristics. Species names are consistent with the catalog of the CCBA collection.

Strain	CCBA designation	Cell volume min.– (median)–max [µm³]	Cell shape
Oocystis submarina	BA-1	42–(92)–285	rotational ellipsoid
Chlorella vulgaris	BA-2	65–(133)–303	sphere
Oocystis parva	BA-3	45–(147)–260	rotational ellipsoid
Chlorella minutussima	BA-12	8–(18)–73	sphere
Chlorella fusca	BA-18	33–(113)–2144	sphere
Chlorella vulgaris	BA-76	15–(35)–103	rotational ellipsoid
Chlorella vulgaris	BA-80	65–(155)–425	sphere
Scenedesmus sp.	BA-147	66–(244)–707	rotational ellipsoid
Dictyosphaerium sp.	BA-157	31–(131)–298	sphere
Monoraphidium sp.	BA-165	34–(67)–120	2 cones
Chlorella sp.	BA-167	19–(50)–126	sphere
Stichococcus sp.	BA-168	5–(12)–24	cylinder
Oocystis cf. *submarina*	BA-172	66–(129)–388	rotational ellipsoid
Microthamnion kuetzingianum	BA-173	48–(131)–237	cylinder; filamentous

Quantitative measurements of lipids were performed using the optimized colorimetric sulfo-phospho-vanillin method (SPV method) by Chabrol & Charonnat (1937). To extract lipids, 1 ml aliquots of algal cultures were centrifuged (10 000 rpm, 5 min) and re-suspended in 0.5 ml of methanol. To fully disrupt the cell wall and to improve the extraction of lipids, glass beads were added and then samples were vigorously shaken for 10 min at 2000 rpm. Subsequently, 1 ml of chloroform was added and the shaking procedure was repeated (Folch et al. 1957). Then, samples were centrifuged, supernatant was collected and supplemented with 0.2 ml of 0.8% NaCl solution. Samples were then left in room temperature until two layers were formed and the upper layer was collected and discarded. The lower layer was dried under N_2 at 50°C. Next, 0.3 ml of concentrated sulfuric acid was added and samples were heated at 90°C for 10 min. After that step, 1 ml of SPV reagent (1.2 g vanillin per 1 liter of 68% phosphoric acid) was added in order to fully stain the fatty acids and the samples were incubated at 36°C for 5 min. The absorbance of the final solution was measured at 525 nm (Knight et al. 1972). The concentration of lipids was estimated based on the calibration curve that was developed using high-quality soybean oil as a source of fatty acids, which has a similar composition of lipids compared to algae (Grama et al. 2014).

The volumetric lipid productivity values of the examined strains were calculated according to the equations recommended by Griffiths & Harrison (2009) and Xu & Boeing (2014):

$$P_{LV} = \mu \times Q$$

where P_{LV} is the volumetric lipid productivity [mg l^{-1} d^{-1}], µ is the specific growth rate [d^{-1}] and Q is the lipid yield [mg l^{-1}].

To compare the mean values of the analyzed parameters, analysis of variance (ANOVA) was performed (Stanisz 2007a). In order to separate the strains with the highest potential for lipid production, cluster analysis was employed, using the agglomerative hierarchical clustering method based on the final cell density, growth rates and lipid yields (Stanisz 2007b). All statistical analyses were performed using Statistica 10 (StatSoft Inc., USA).

Results and discussion

Growth rate and final cell number

All strains showed a rapid increase in the number of cells during the 21-day period of culture (Table 2). There was no case of growth inhibition or decline in the number of cells. Strains belonging to the *Chlorella* genus exhibited the highest growth rate, ranging from 0.39 d^{-1} to 0.5 d^{-1}, and thus the highest final cell number (up to 3.72×10^7 cells ml^{-1}). The results correspond to growth rates obtained for other *Chlorella* strains, e.g. Kong et al. (2011) reported the growth rate at the level of 0.32 d^{-1} in the phototrophic culture. Remaining strains exhibited a lower growth rate, varying within the range of 0.27 d^{-1} and 0.38 d^{-1}. Differences observed in species-specific

Table 2

Growth and lipid accumulation characteristics in the tested strains

Strain	Final cell count [cells ml^{-1}]	Growth rate μ [d^{-1}]	Lipid yield [mg l^{-1}]	Cellular lipid content [pg cell^{-1}]
Oocystis submarina BA-1	$3.56 \times 10^6 \pm 7.72 \times 10^4$	0.35 ± 0.01	57.59 ± 0.50	16.19 ± 0.29
Chlorella vulgaris BA-2	$1.13 \times 10^7 \pm 2.66 \times 10^5$	0.42 ± 0.01	56.82 ± 6.29	5.05 ± 0.43
Oocystis parva BA-3	$7.25 \times 10^6 \pm 1.53 \times 10^5$	0.38 ± 0.01	38.64 ± 2.17	5.33 ± 0.33
Chlorella minutussima BA-12	$3.72 \times 10^7 \pm 7.25 \times 10^6$	0.5 ± 0.06	62.52 ± 9.78	1.68 ± 0.26
Chlorella fusca BA-18	$1.64 \times 10^7 \pm 3.85 \times 10^5$	0.45 ± 0.01	64.85 ± 10.81	3.96 ± 0.64
Chlorella vulgaris BA-76	$1.33 \times 10^6 \pm 7.83 \times 10^5$	0.26 ± 0.03	3.50 ± 3.13	2.63 ± 2.51
Chlorella vulgaris BA-80	$3.07 \times 10^7 \pm 1.44 \times 10^6$	0.49 ± 0.01	75.90 ± 9.32	2.48 ± 0.41
Scenedesmus sp. BA-147	$1.87 \times 10^6 \pm 2.02 \times 10^5$	0.26 ± 0.01	46.39 ± 8.49	24.81 ± 2.70
Dictyosphaerium sp. BA-157	$9.84 \times 10^6 \pm 3.71 \times 10^5$	0.30 ± 0.01	31.57 ± 3.44	3.21 ± 0.15
Monoraphidium sp. BA-165	$1.30 \times 10^7 \pm 3.65 \times 10^5$	0.35 ± 0.01	81.13 ± 15.91	6.26 ± 1.29
Chlorella sp. BA-167	$1.65 \times 10^7 \pm 2.64 \times 10^5$	0.39 ± 0.01	80.53 ± 3.60	4.89 ± 0.13
Stichococcus sp. BA-168	$1.95 \times 10^7 \pm 3.38 \times 10^6$	0.35 ± 0.01	16.80 ± 4.34	0.86 ± 0.15
Oocystis cf. *submarina* BA-172	$2.80 \times 10^6 \pm 9.05 \times 10^4$	0.24 ± 0.01	43.47 ± 7.29	15.54 ± 2.90
Microthamnion sp. BA-173	$4.61 \times 10^6 \pm 1.70 \times 10^5$	0.30 ± 0.01	56.41 ± 2.74	12.23 ± 0.14

growth rates may be explained by the fact that the growth of unicellular microalgae is inversely proportional to their cell size (Banse 1976). This was also observed in the present study. The strains with the highest cell volume, e.g. *Scenedesmus* sp. BA-147, showed a rather low growth rate and thus a lower final cell count compared to small-sized species, e.g. *Chlorella minutussima* BA-12, the volume of which was ca. 20 times smaller than *Scenedesmus* sp. BA-147 (Tables 1, 2). This is due to the fact that larger cells exhaust metabolic energy more quickly, which results in slower growth (Borowitzka 1992). The size of a cell also determines its surface area to volume ratio (SA:V ratio). This consequently affects the efficiency of nutrient uptake by cells; small cells with the higher SA:V ratio assimilate nutrients more effectively (Fogg 1975). The opposite effect is observed in larger cells, where also the supply of various substances from the cell surface to its interior through diffusion is less effective (Nielsen 2006). Consequently, size-dependent nutritional properties of microalgae regulate their overall metabolism and hence their growth rate (Fogg 1975; Hein et al. 1995).

Furthermore, it was also proved that there are size-dependent maximal densities of cells in microalgal cultures, regardless of their type (Agustí et al. 1987). The study showed that algae with larger cells grew more slowly and maintained lower biomass due to the extinction of light caused by the cells themselves. This phenomenon, called self-shading, was by far the most important limiting factor. The growth form of microalgae also affects their growth rates and thus colony-forming species with larger cells may reach densities close to those observed in smaller species. In the set of tested microalgae, the strain of colonial *Dictyosphaerium* sp. BA-157 grew well, reaching high final cell density of 9.84×10^6 cells ml^{-1} and being among the most abundant strains of similar size (Table 2). The research showed that there is no clear relationship for the size-dependent maximum growth of microalgae growing in colonies, because they are a form of evolutionary adaptation that allows avoiding constraints resulting from increasing cell size (Nielsen 2006).

Rapid growth rates are considered an important feature of microalgal species cultured for commercial purposes, providing a competitive edge over microorganisms (including other algal species) that cause contamination of outdoor cultures. Small-sized fast-growing microalgae also require less culture space due to the higher cell density (Tan & Lee 2016). Whereas considering the forms of algal growth, species exhibiting the colonial growth are able to reduce grazing pressure (Nielsen 2006).

Cellular lipid content, volumetric lipid yield and productivity

Significantly higher cellular lipid content was observed in 4 strains with the highest cell volume, i.e. *O. submarina* BA-01 (16.19 pg cell^{-1}), *Scenedesmus* sp. BA-147 (24.81 pg cell^{-1}), *O.* cf. *submarina* BA-172 (15.54 pg cell^{-1}) and *Microthamnion* sp. BA-173 (12.23 pg cell^{-1}) ($p < 0.05$, Fisher LSD test), while in other strains it ranged from 1.68 pg cell^{-1} in *C. minutussima* BA-12 to 6.26 pg cell^{-1} for *Monoraphidium* sp. BA-165. The cellular lipid content patterns among the strains were not reflected in the final lipid yield values as they also depend on the growth rate of the strain. In the 21st day of culture, lipid yields varied within the range of

3.50 mg l^{-1} (*Chlorella vulgaris* BA-76) and 81.13 mg l^{-1} (*Monoraphidium* sp. BA-165). The comparison of values obtained for all tested strains enabled the identification of cultures with significantly higher final lipid yields, i.e. *C. minutissima* BA-12 (62.52 mg l^{-1}), *C. fusca* BA-18 (64.85 mg l^{-1}), *C. vulgaris* BA-80 (75.90 mg l^{-1}), *Chlorella* sp. BA-167 (80.53 mg l^{-1}) and *Monoraphidium* sp. BA-165 (81.13 mg l^{-1}) ($p < 0.05$, Fisher LSD test), which, as mentioned earlier, were also characterized by high growth rates and final cell densities (Table 2).

Griffiths & Harrison (2009) showed the volumetric lipid productivity (P_{LV}) as a universal parameter and a key characteristic when choosing algal species for valuable biomass production. It combines the growth rate of strains (calculated for the exponential growth phase) and volumetric lipid yield, giving easily comparable results among various microalgae strains. Fig. 1 presents productivity rates for all tested strains. Among them, five strains belonging to the *Chlorella* genus, namely BA-02, BA-12, BA-18, BA-80, BA-167, and *Monoraphidium* strain BA-165 showed significantly higher productivity values, varying within the range of 23.86 and 37.42 mg l^{-1} d^{-1} (Fisher LSD test; $p < 0.05$). The *C. vulgaris* BA-80 strain was marked with the top lipid productivity of 37.42 mg l^{-1} d^{-1} and high growth rate (0.49 d^{-1}). In the second most productive strain, i.e. *Chlorella* sp. BA-167, the growth rate was lower (0.39 d^{-1}), resulting in lower lipid productivity.

Although the volumetric lipid productivity is a very useful parameter in assessing the potential of microalgal strains for the mass culture and lipid production, it is advisable to compare the calculated values with other parameters such as the lipid yield after a certain period of culture, but before the full stationary phase (Wood et al. 2005; Borowitzka 2013; Xu & Boeing 2014). In this study, both parameters indicated the five strains as the most promising ones for biomass production, i.e. *C. minutussima* BA-12, *C. fusca* BA-18, *C. vulgaris* BA-80, *Monoraphidium* sp. BA-165 and *Chlorella* sp. BA-167.

The range of lipid productivity recorded in the literature covers values that differ by several orders of magnitude (Table 3), reaching up to 200 mg l^{-1} d^{-1} (Nascimento et al. 2013). There are several-fold differences among various microalgal species and strains grown in laboratory batch cultures. For instance, the green alga *Botryococcus braunii*, often marked with the highest lipid yields, was characterized by considerable variations with respect to its growth rate and productivity (5.51 vs. 112.43 mg l^{-1} d^{-1}), resulting from the *B. braunii* strain-specific characteristics, the physiological state of strains as well as culture conditions (Metzger & Largeau 2005; Tasi et al. 2016). Productivity of most of the selected

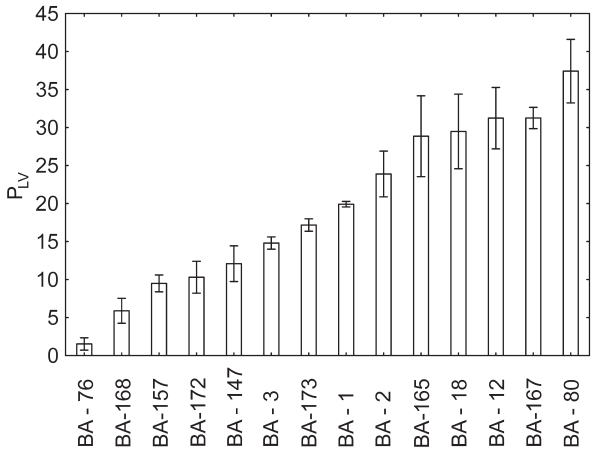

Figure 1

Volumetric lipid productivity (PLV) in the tested Baltic green algae strains

Baltic strains tested in this study fits well within the range of P_{LV} reported in the literature (Table 3). Taking into account the tested strains of the *Chlorella* genus, it was shown that for some of them, i.e. BA-2, BA-12, BA-80 and BA-167, the lipid productivity rates were similar to those observed in other research screening microalgal strains for most efficient producers of lipids (Table 3). For instance, Lee et al. (2010) recorded *Chlorella* strain productivity at the level of 11.1 mg l^{-1} d^{-1}, although at slightly higher light intensity, i.e. 150 µmol m^{-2} s^{-1}, and aeration of the culture medium. When culturing C. *minutussima*, Illman et al. (2000) also recorded the production of lipids around 10 mg l^{-1} d^{-1}, but at the lower light intensity (77 µmol m^{-2} s^{-1}) and with considerably higher temperature (25°C). Whereas Griffiths et al. (2012) determined almost three times higher productivity rate, 27 mg l^{-1} d^{-1}, for *C. vulgaris* grown at 25°C and light intensity of 250 µmol m^2 s^{-1} in the medium enriched with CO_2. In this study, the P_{LV} values estimated for the most productive *Chlorella* strains under relatively lower temperature (compared to other screening studies for high lipid content strains) were around 30 (up to 37) mg l^{-1} d^{-1}, falling within the upper range of microalgal lipid productivity compared to literature data (see Table 3). The presence of *Monoraphidium* sp. BA-165 among the strains with the highest P_{LV} values is also not surprising as previous studies have already confirmed the potential of this genus for increased production of lipids (Yu et al. 2012; Bogen et al. 2013).

To identify the most promising strains for mass culture, cluster analysis based on growth rates, final cell density and lipid yields was carried out. The tested strains were grouped into two main groups, each of which was then divided into two specific

Table 3

Volumetric lipid productivity (PLV) of green algae reported in literature, listed together with Baltic strains indicated in this study as characterized by high productivity

Species/strain	P_{LV} [mg × l^{-1} × day^{-1}]	Culture conditions temp.; light; medium	References
Botryococcus braunii	5.51	25°C; 150 µmol m^2 s^{-1}; CHU13	(Yoo et al. 2010)
Monoraphidium sp. FXY-10	6.88	25°C; 70 µmol m^2 s^{-1}; BG11	(Yu et al. 2012)
Chlorella vulgaris	6.91	25°C; 150 µmol m^2 s^{-1}; BG11	(Yoo et al. 2010)
Scenedesmus sp.	9.50	150 µmol m^2 s^{-1}	(Lee et al. 2010)
Chlorella minutussima	10.24 (calculated)	25°C; 77 µmol m^2 s^{-1}; Guillard's marine medium	(Illman et al. 2000)
Chlorella vulgaris	11.10	150 µmol m^2 s^{-1}; BG11	(Lee et al. 2010)
Chlorococcum sp.	11.30	28°C; 54 µmol m^2 s^{-1}; BG11	(Harwati et al. 2012)
Botryococcus sp.	11.50	150 µmol m^2 s^{-1}; BG11	(Lee et al. 2010)
Microthamnion sp. BA-173	17.23	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Oocystis submarina BA-1	19.92	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Scenedemus sp.	20.65	25°C; 140 µmol m^2 s^{-1}; CHU13; CO_2 enriched	(Yoo et al. 2010)
Chlorella vulgaris BA-2	23.89	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Kirchneriella lunaris	24.22	25°C; 140 µmol m^2 s^{-1}; CHU13; CO_2 enriched	(Nascimento et al. 2013)
Scenedesmus obliquus	26.77	25°C; 140 µmol m^2 s^{-1}; CHU13; CO_2 enriched	(Nascimento et al. 2013)
Chlorella vulgaris	27.00	25°C; 250 µmol m^2 s^{-1}; 3N BBM; CO_2 enriched	(Griffiths et al. 2012)
Monoraphidium sp. BA-165	28.85	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Scenedesmus sp.	29.00	25°C; 250 µmol m^2 s^{-1}; 3N BBM; CO_2 enriched	(Griffiths et al. 2012)
Chlorella fusca BA-18	29.49	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Chlorella minutussima BA-12	31.23	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Chlorella sp. BA-167	31.26	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Chlorella vulgaris BA-80	37.43	18°C; 100 µmol m^2 s^{-1}; f/2	this study
Ankistrodesmus falcatus	56.07	25°C; 140 µmol m^2 s^{-1}; CHU13; CO_2 enriched	(Nascimento et al. 2013)
Botryococcus braunii	112.43	25°C; 140 µmol m^2 s^{-1}; CHU13; CO_2 enriched	(Nascimento et al. 2013)
Chlorella vulgaris	204.91	25°C; 140 µmol m^2 s^{-1}; CHU13; CO_2 enriched	(Nascimento et al. 2013)

branches, indicating strains with similar characteristics (Fig. 2). The upper branch grouped species tentatively described as those having low potential for efficient mass culture to produce lipids; the strains were characterized by low to moderate growth rates and yields of lipids (Table 2). Whereas the second group contained strains that could be defined as very promising for the production of lipids. Within this group, two specific clusters were distinguished: one containing two strains, *Monoraphidium* sp. BA-165 and *Chlorella* sp. BA-167, revealing the highest lipid yield and the second one containing four strains, *C. vulgaris* BA-02, *C. minutussima* BA-12, *C. fusca* BA-18 and *C. vulgaris* BA-80, which were characterized by the highest values of the volumetric lipid productivity, i.e. the highest growth rate and only slightly lower lipid yield (Table 2). In this study, temperature, light intensity and salinity conditions were set to enable the uninhibited growth of microalgae, but not necessarily to obtain their highest possible efficiency. Therefore, there is still plenty of scope for improvement and stimulation of productivity to reach much higher lipid yield values, e.g. higher temperature, stronger light, CO_2 enrichment or stress factors such as nitrogen limitation or salinity stress (Courchesne et al. 2009). Referring to Table 3, a significant increase in microalgal productivity was observed with CO_2-enriched aeration and at higher temperature, indicating their possible influence. This confirms the need for further research on the conditions of lipid productivity stimulation, affecting local strains, because algal strains of the

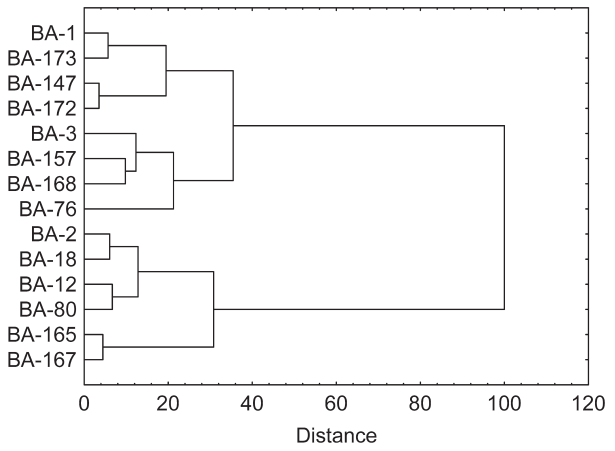

Figure 2

Cluster analysis of the tested Baltic green algae strains based on their growth rates and lipid yield data

same species have different biochemical profiles and stress responses, depending on the natural habitat conditions and phenotype plasticity in a single genotype (Miner et al. 2005). A recent study confirmed that brackish water species have slightly higher lipid content under stress compared to the typical marine species, which makes Baltic strains even more promising in biotechnological applications (Schwenk et al. 2013).

Conclusion

Fourteen selected Baltic green algae strains manifested different growth rates with a clear effect of cell size. Strains belonging to the *Chlorella* genus exhibited the highest growth rate, ranging from 0.39 d^{-1} to 0.50 d^{-1}, and thus they also showed the highest final cell number. Five strains showed the highest lipid yield [mg l^{-1}], i.e. *C. minutissima* BA-12 (62.52 mg l^{-1}), *C. fusca* BA-18 (64.85 mg l^{-1}), *C. vulgaris* BA-80 (75.90 mg l^{-1}), *Chlorella* sp. BA-167 (80.53 mg l^{-1}) and *Monoraphidium* sp. BA-165 (81.13 mg l^{-1}). For the same strains, including additionally the strain *C. vulgaris* BA-02, the highest volumetric lipid productivity values were determined. The P_{LV} values of the majority of strains tested in this study fitted within the upper range of lipid productivity rates recorded in the literature, i.e. 10–40 mg l^{-1} d^{-1}. Such rates were obtained under culture conditions allowing for stable and uninhibited growth in relatively low temperature, leaving a broad spectrum of possibilities for cell growth improvement and stimulation. Regarding the fact that the production of lipids can be stimulated by various stress conditions, it is possible to significantly improve the productivity of lipids in the examined strains, which encourages further exploration of the potential of Baltic brackish strains to produce biomass rich in lipids.

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